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Physiology and Genetics of the "Ribonucleic Acid Control" Locus in *Escherichia coli*

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INTRODUCTION

Amino Acid Control of Ribonucleic Acid (RNA) Synthesis

In 1952, Sands and Roberts (87) reported the results of a study on the relation between the synthesis of proteins and of nucleic acids. They showed that when a culture of a strain of *Escherichia coli* that required tryptophan and histidine was deprived of either amino acid, not only did protein synthesis stop, but there was also a reduction in the rate of RNA synthesis. They inferred that, since neither tryptophan nor histidine is a metabolic precursor of nucleic acid, the reduction in RNA synthesis was a secondary effect of the primary blockage in protein synthesis. The important conclusion drawn was that there is a regulatory connection between the synthesis of proteins and of nucleic acids. These observations were confirmed and extended by other workers (36, 42, 43, 74). The original hypothesis was that this regulation was effected by transfer RNA (tRNA) (48, 94), and it was then proposed (40) that uncharged tRNA inhibited the RNA polymerase, and that such inhibition resulted in the reduced synthesis of all classes of RNA.

One exception to the rule that starvation of a bacterium for a required amino acid stops not only protein synthesis but also RNA synthesis was the finding (7, 8) that, in the methionine-requiring strain W6 of *E. coli* K-12, RNA synthesis continues in the absence of methionine (*see* Fig. 1). This strain was referred to by Stent and Brenner (94) as having a "relaxed" control of RNA synthesis, in contrast to the "stringent" control that normally obtains. It was shown that the property of stringent or relaxed control of RNA synthesis maps as a single genetic locus, the RNA control or "RC" locus.

The object of this review is to relate the various observations on the nature of the regulatory connection between protein synthesis and RNA syn-

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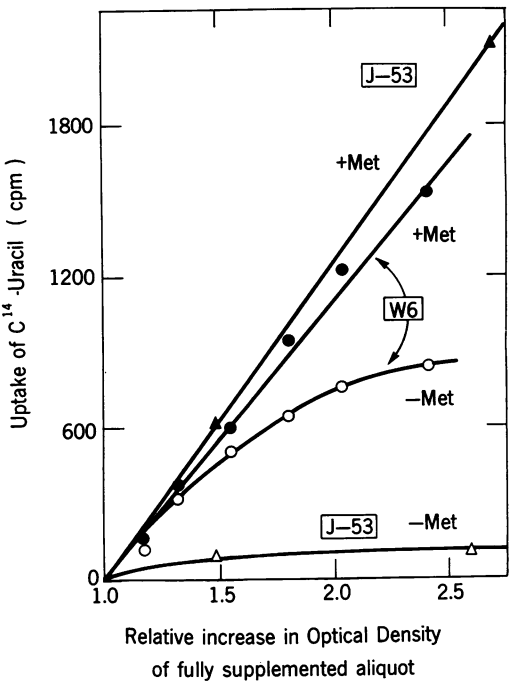

FIG. 1. *Effect of methionine starvation on the synthesis of RNA. The incorporation of radioactive uracil into the acid-insoluble fraction of two different methionine auxotrophs, strains J-53 and W6, was followed in the presence and in the absence of methionine. Strain J-53 has a stringent control of RNA synthesis, whereas in strain W6 this control is relaxed. Redrawn from Stent and Brenner (94).*

thesis, and to evaluate the theories on the mechanism of this regulation that have so far been advanced.

Nomenclature

Various symbols have been used to represent strains with stringent and with relaxed control of RNA synthesis. In this review, we shall use the symbol *rel* to represent the genetic locus determining this function. This symbol, unlike "RC," conforms to the rules advocated by Demerec et al. for representing genotypes (19) and has already been used for this purpose (98). The mutation present in strain W6, being the one defining this locus, is designated *rel-1*. In the absence of extensive complementation data, it is as yet not possible to decide if all such mutations fall within the same cistron.

In the system of Demerec et al. (19), the phenotype symbol is clearly distinct from the genotype symbol. The symbols RC^{str} and RC^{rel}, for stringent and relaxed control of RNA synthesis, respectively, can legitimately be used to describe phenotype. Since they are unambiguous and widely used, we urge their continued usage in describing the phenotypes engendered by the wild-type and mutant alleles of the *rel* gene.

PHYSIOLOGICAL STUDIES

Amino Acid Starvation and RNA Synthesis

Amino acid starvation. The principal physiological difference between RC^{str} and RC^{rel} strains is manifested under conditions of effective amino acid starvation, when RC^{rel} but not RC^{str} strains continue to accumulate RNA. In amino acid-starved RC^{str} strains, the rate of residual RNA synthesis is reduced to a few per cent of that found in the presence of the required amino acid, whereas amino acid-starved RC^{rel} strains continue to produce RNA at normal or slightly higher than normal rates (94). Within these limits, considerable variation can occur in the rate of residual RNA synthesis, as will be discussed presently.

To demonstrate the difference in the rate of residual RNA synthesis between RC^{str} and RC^{rel} strains, bacteria can be amino acid-starved in several different ways. Perhaps the most frequently used technique involves the use of auxotrophs which require an exogenously supplied amino acid for growth. Removal of the amino acid from the medium causes an immediate cessation of protein synthesis in RC^{str} and RC^{rel} strains. Another technique involves the use of amino acid analogues, such as β-2-thienylalanine, 2-thiazolealanine, and 5-methyltryptophan (81). These analogues interfere with the synthesis of the corresponding natural amino acid and therefore create a situation of amino acid starvation. Certain strains of *E. coli*—for instance strain K-12 but not strain B—can be starved for isoleucine by addition of valine, which interferes with isoleucine biosynthesis by feedback inhibition of the first enzyme in the isoleucine biosynthetic pathway (78). Amino acid starvation resulting from the addition of such inhibitory compounds to bacterial cultures is useful for distinguishing between the RC^{str} and RC^{rel} phenotypes, since it makes possible amino acid starvation of bacteria that have no amino acid requirements and because it is convenient for handling large numbers of test cultures.

A third technique which creates a condition of amino acid starvation involves a "shift-down," or transfer of a bacterial culture, from a medium containing a complete complement of amino acids to a minimal medium containing only

nutrients required for growth. Since the repression of the enzymes of amino acid biosynthesis in the complete medium results in a state of "physiological auxotrophy," the shiftdown creates a transient condition of amino acid starvation.

RNA synthesis. The rate of residual RNA synthesis under these conditions of amino acid starvation can be measured by chemical estimation of the total amount of RNA. This method measures net rather than total synthesis of RNA, since a situation in which RNA is synthesized and then broken down is not detected. RNA synthesis can also be measured by following the incorporation of a radioactive precursor of RNA into trichloroacetic acid-precipitable material. Such incorporation may be detected even in the absence of net RNA synthesis, again because of turnover of unstable RNA. It must also be kept in mind that failure to observe incorporation can be ascribed to causes other than cessation of RNA synthesis, for instance to the failure to take up the precursor from the medium or to convert it to a substrate suitable for incorporation into RNA. Thus it has been demonstrated that, during amino acid starvation, RC^{str} bacteria assimilate and phosphorylate exogenously supplied pyrimidines much less readily than do RC^{rel} bacteria (21). In this particular case, however, it is likely that there is a connection between reduction in the rate of RNA synthesis and in uptake of pyrimidines from the medium, although the nature of this connection is not clear.

To date, various auxotrophic strains have been starved for most of the 20 standard amino acids (32, 94), with the general result that, upon starvation, RNA synthesis ceases in RC^{str} strains and continues in RC^{rel} strains. Fiil and Friesen (32) showed that in each of a series of independently isolated *rel* mutants, all derived from the same rel^+ parent strain, the amount of radioactive uracil incorporated during starvation for different amino acids (threonine, leucine, arginine, and histidine) was essentially the same. The only anomalous datum (3) so far has been the observation that RNA synthesis ceases upon starvation of a serine/gycine$^-$ *rel-1* strain. This anomaly does not simply result from the role of glycine as a precursor of purine biosynthesis, since the presence of adenine in the medium during starvation does not restore RNA synthesis.

Two instances of variation in the rate of radioactive uracil incorporation on amino acid starvation have been described. The first is in the series of strains isolated by Fiil and Friesen (32). They demonstrated that the rate of incorporation during amino acid starvation varies widely between strains and is characteristic for each mutant. They concluded that these strains differ in the amount of RNA that is synthesized during amino acid starvation. In the other case, considerable variation in the amount of uracil incorporation was observed (3) among Arg$^-$ recombinants of a cross between an Hfr strain carrying the *rel-1* mutation and a rel^+ F^- strain. Despite the wide range in the amount of uracil incorporation, recombinants could be clearly distinguished as RC^{str} or RC^{rel}. It must be concluded, therefore, that genes other than the *rel* locus may have an effect on this operational test of the residual rate of RNA synthesis in amino acid-starved bacteria.

Types of regulation of RNA synthesis. A clear distinction should be made between the control of RNA synthesis shown by RC^{str} strains under amino acid starvation and the regulation of the rate of RNA synthesis during normal growth. In growing cultures, the number of ribosomes per bacterium increases with increasing growth rate, as does tRNA (54). An exception to this rule is that, at low growth rates, tRNA is synthesized in relative excess (85). That is, bacteria are able to adjust the synthesis of these two classes of RNA to a rate commensurate with the growth rate.

The regulatory aberration of RC^{rel} strains manifests itself only during amino acid starvation, for, as demonstrated by Neidhardt (68), RC^{rel} strains *are* able to regulate RNA synthesis when subjected to shifts in carbon or nitrogen source which either enhance or depress the rate of overall growth. The ability of RC^{rel} strains to adjust their rate of RNA synthesis with growth rate has been confirmed with an isogenic pair of RC^{str} and RC^{rel} strains (G. Edlin, *unpublished data*). These results emphasize the fact that the *rel* locus affects RNA synthesis only during amino acid starvation and that the general regulation of RNA synthesis during exponential growth in RC^{rel} strains is indistinguishable from that of RC^{str} wild-type strains.

Consequences of RC^{rel} Expression

Chloramphenicol particles and relaxed particles. When bacteria are treated with chloramphenicol, protein synthesis is inhibited but RNA synthesis continues. The continuation of RNA synthesis in the absence of protein synthesis produces an accumulation of "chloramphenicol particles" which have been presumed to be ribosomal precursors, since the RNA encapsulated in these particles can be matured into complete, functional ribosomes when protein synthesis is restored by removal of the chloramphenicol. An analogous situation obtains during amino acid starvation of an RC^{rel} strain. Here, too, RNA synthesis continues in the absence of protein synthesis, resulting in an accumulation of "relaxed particles." Because of the information these particles may

provide in understanding the many steps in ribosomal biosynthesis, their synthesis and composition have been extensively studied. A considerable literature exists on the properties of these particles (16, 49, 58, 67, 97), but, since these studies do not bear directly on the nature of the *rel* mutation, we shall not discuss them further in this review.

Permeability effects. There exist mutant strains of *Bacillus* and closely related genera and of *E. coli* that excrete substantial amounts of glutamate. In general, these mutant strains have a requirement for biotin, and the optimum condition for glutamate excretion is that of biotin-limited growth (18). Two biotin-requiring strains of *E. coli* grown under conditions of biotin limitation have been reported to have a lipid deficiency in the membraneous components of the cell (15, 38). It has been proposed that this lipid deficiency results in a permeability defect which allows glutamate to escape from the cell. In the absence of an effective feedback control, the production and excretion of glutamate continues.

On amino acid starvation of an RC^{rel} strain but not an RC^{str} strain, glutamate is also excreted (P. Broda, *in preparation*). The rate of glutamate excretion gradually increases after the onset of amino acid starvation, reaching a maximum after about 30 min at 37 C. It is therefore proposed that, during the relaxed response, there is an alteration in cellular permeability which becomes fully expressed at that time, allowing continued synthesis and excretion of glutamate. This hypothesis is consistent with the finding that RC^{rel} strains of *E. coli* become somewhat permeable to actinomycin D during amino acid starvation (H. Matzura and P. Broda, *in preparation*).

Amino acid sensitivities. In an amino acid shift-down, a situation of temporary amino acid starvation is created and persists until the bacteria have produced the amino acid biosynthetic enzymes. RC^{rel} cells take much longer to recover from such a shift-down than RC^{str} cells (3, 68), and this response of RC^{rel} cells to a shift-down depends upon their stage of growth (3). Appropriate dilutions from a broth-grown culture of an RC^{rel} methionine-requiring strain in various stages of growth were plated onto tryptone-broth plates and onto minimal agar supplemented with methionine. Whereas the colony counts on the broth-agar were a measure of the culture's growth, the minimal agar colony counts, although coincident with the broth-agar colony counts in the stationary phase of growth, were as low as 20% of the broth values for cells at the beginning of the exponential phase of growth. It appears, therefore, that RC^{rel} cells are especially sensitive to a shift-down at this stage. This shift-down sensitivity is manifested in a dramatic fashion by the addition of 500 μg/ml of leucine to the minimal agar. The percentage of survivors from an RC^{rel} culture at the beginning of the exponential phase of growth is now less than 0.1, and cells at all stages of growth, including those from the final nongrowing culture, are sensitive to some degree.

TABLE 1. *Amino acid sensitivities of recombinants from a cross of the type Hfr RC^{rel} × F^- RC^{str}* [a]

Phenotype	+ Leucine		+ Methionine		+ Phenylalanine	
	Sensitive	Insensitive	Sensitive	Insensitive	Sensitive	Insensitive
RC^{rel}	35	0	35	0	18	17
RC^{str}	0	56	0	56	0	56

[a] Hfr strain Cavalli (Met^- RC^{rel} leucine-sensitive methionine-insensitive phenylalanine-insensitive) was crossed with an F^- strain PA309 (Leu^- His^- Arg^- RC^{str} leucine-insensitive methionine-insensitive phenylalanine-insensitive). Leu^+ Met^+ His^+ Arg^- recombinants were tested for the RC phenotype and for their amino acid sensitivities (data from reference 3).

Recombinants from a conjugational cross between an RC^{rel} leucine-sensitive Hfr strain and a leucine-insensitive RC^{str} recipient were analyzed (3) for the linkage between the leucine-sensitivity property and the RC property (Table 1). There was a complete correlation, in that all the RC^{str} recombinants were insensitive and all the RC^{rel} recombinants were sensitive.

Other sensitivities which at least partially correlated with the RC^{rel} phenotype were observed on the addition of phenylalanine and of methionine. Both parent strains used in the conjugation experiment described above were insensitive to these amino acids; however, all the RC^{rel} recombinant clones, but none of the RC^{str} clones, were sensitive to methionine (Table 1). Also, there were phenylalanine-sensitive clones among the RC^{rel} recombinants but not among the RC^{str} ones. The conclusion was that RC^{rel} bacteria tend to be more sensitive than RC^{str} cells to some amino acids in the shift-down condition. The sensitivity to leucine and phenylalanine can be relieved by the addition of mixtures of leucine, isoleucine, and valine, suggesting that here, as in valine sensitivity, inhibition is caused by a derangement of the system which regulates the synthesis of leucine, valine, isoleucine, and threonine (1). Sensitivity to a high external concentration of an amino acid, leading to such a derangement, could occur in an RC^{rel} strain but not in an RC^{str} strain if, on amino acid starvation, the

RC^{rel} strain were to become more permeable than the RC^{str} strain.

RC^{rel} phenotype and conjugation. The RC^{rel} phenotype might be a complicating factor in the analysis of conjugation experiments. The yield of recombinants obtained in a genetic cross depends both upon the medium in which the mating is performed and the constitution of the agar plates on which the recombinants are selected. The lowest yields have been obtained with matings in broth or Casamino Acids medium followed by plating on minimal agar plates (44, 82); these are, in fact, shift-down situations. The presence of the RC^{rel} property might then influence both the yield and the types of recombinants isolated in crosses, especially when the recipient strain is RC^{rel}. It happens that both Hfr strains, Cavalli and Hayes, were isolated from an F^+ RC^{rel} strain, so that many genetic analyses have been done on recombinants of crosses of the type Hfr RC^{rel} × F^- RC^{str}.

GENETICS

Mapping of the rel Locus

Bacterial conjugation experiments showed that the *rel* locus is located between the *strA* and *glyA* loci on the *E. coli* chromosome (2). More refined transductional mapping with phage P1 has indicated that the *rel* locus lies between the *argA* and *cysC* loci, or between minutes 53 and 54 of the Taylor-Trotter map (98). An analogous mutation in *Salmonella typhimurium* is reported to map in a similar position in that species (60).

A large number of RC^{rel} strains of independent origin have been isolated from RC^{str} strains by Fiil and Friesen (32) and by Lavallé (50). The mutations carried by most of these mutant strains map in the same region as the *rel* locus. However, in three of Lavallé's isolates, the mutation does not map in the *rel* region (50). These three isolates, although of RC^{rel} phenotype, in the sense that RNA accumulates in the absence of a required amino acid, have some physiological properties which distinguish them from strains with the classical RC^{rel} phenotype.

Selection of Mutants and Transductants

Early comparative investigations of the properties of RC^{rel} and RC^{str} strains were done with nonisogenic strains. However, four procedures have been developed to obtain closely related pairs of RC^{str} and RC^{rel} strains. The first of these procedures is that of penicillin selection (32); it depends upon the observation that glucose-grown RC^{rel} bacteria, which have been extensively starved for a required amino acid in the presence of glucose and subsequently shifted to fresh medium containing lactose as the carbon source together with the required amino acid, exhibit a much greater growth lag than a similarly treated RC^{str} strain (3). If penicillin is present in this lactose medium, the RC^{str} bacteria will be selectively killed before the RC^{rel} bacteria begin multiplying. If the penicillin is removed prior to the onset of growth of the RC^{rel} bacteria, a culture enriched in RC^{rel} bacteria is obtained. It was found that mutagenesis of a bacterial culture, followed by five cycles of penicillin selection, was sufficient to allow the isolation of RC^{rel} bacteria. It should also be possible to isolate RC^{str} derivatives of an RC^{rel} strain by making use of the earlier resumption of growth by RC^{str} strains after such a glucose-lactose shift. The advantage of the penicillin method lies in the opportunity of selecting independently arising mutants from a given parental strain, so that different mutants can be compared. The major defect in this procedure is that it is not known how many mutations may have occurred in the formation of the phenotypically "relaxed" strain that is isolated.

A second procedure, developed by Lavallé (quoted in 32), involves transduction. It is impractical to select directly for RC^{str} or RC^{rel} transductants; therefore, the desired *rel* allele is introduced as a joint transductant with the closely linked thymine marker *thyA*. This method relies on the prior selection of a *thyA* mutation in the recipient strain into which the desired *rel* allele is to be introduced by incubation of the Thy^+ wild strain in the presence of aminopterin or trimethoprim (91). Since the cotransduction frequency of *thyA* with *rel* is only a few per cent, a convenient refinement employs a lysate of phage P1 or phage 363 prepared from a donor strain of the genotype $thyA^+argA$ and carrying the *rel* allele to be transferred (32,). This phage lysate is then used to infect the $thyAargA^+$ recipient, and Thy^+ transductants are then selected by plating on thymine-free, arginine-supplemented agar. The Thy^+ transductants are examined by replica plating for clones that have acquired the donor *argA* allele. These $thyA^+argA$ transductants are then tested for the presence of the RC^{rel} or RC^{str} character; the cotransduction frequency of *argA* and *rel* is of the order of 25% (32). An advantage of this method is that it is possible to use the arginine requirement for the test of the RC phenotype, so that RNA synthesis in different strains can be compared under the standard condition of arginine starvation.

The observation that RC^{rel} or RC^{str} strains can be prepared by transduction supports the conclusion that a small region of the *E. coli* chromosome is relevant to the expression of this phenotype (2). Fiil and Friesen have shown, further-

more, that the mutational event involved in the origin of the penicillin-selected RC^{rel} mutants is also confined to this small region of the genome. Lysates of the transducing phage 363 were prepared from a series of RC^{rel} strains isolated by the penicillin technique. These lysates were used to infect an *argA* RC^{str} strain, and *argA*$^+$ transductants were selected. It was found that in each case a proportion of the *argA*$^+$ transductants were of RC^{rel} phenotype and that, on amino acid starvation, the transductants exhibited the same kinetics of RNA synthesis as the original donor strains. It was concluded that the "degree of relaxedness" reflects the particular *rel* mutation.

The third procedure was developed by R. E. MacDonald (*personal communication*); it exploits a morphological difference between colonies of amino acid-starved clones of RC^{str} and RC^{rel} bacteria. Several thousand RC^{str} amino acid auxotrophs are spread on minimal agar which contains only about 1 μg/ml of the required amino acid, and incubated for 24 to 48 hr. Microcolonies appear, but their continued growth is restricted because of the amino acid limitation. The colonies of RC^{str} bacteria cease RNA synthesis at the time of amino acid exhaustion. Colonies of RC^{rel} bacteria, however, continue to synthesize RNA for a period after amino acid exhaustion. This difference in residual RNA synthesis is reflected in the colonial morphology, so that RC^{str} and RC^{rel} colonies can be distinguished by phase contrast microscopy. Figure 2 illustrates this difference.

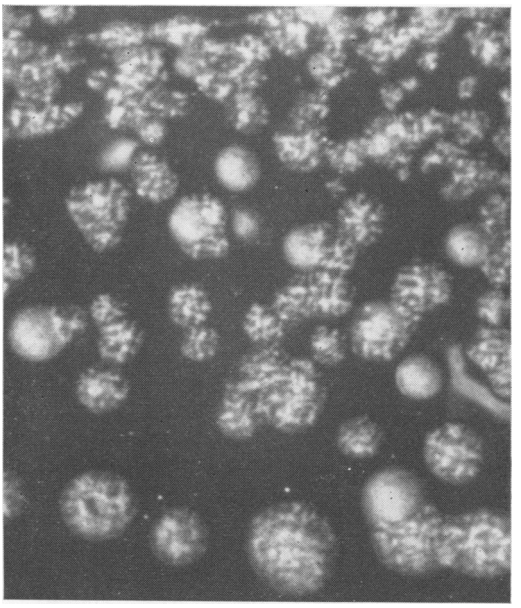

FIG. 2. *Morphological difference between RC^{str} and RC^{rel} strains. A mixture of the RC^{str} strain CP78 and its RC^{rel} derivative CP79 (31) was plated on minimal agar medium containing limiting amounts of a required amino acid. Colonies are observed with a low-power microscope with intense oblique lighting at an angle of about 70° from below. Colonies of the RC^{rel} strain have a mottled appearance, whereas colonies of the RC^{str} strain diffuse light more evenly and are somewhat more opaque.*

An analogous procedure was employed by Martin (60) to isolate an RC^{rel} derivative of a histidine-requiring strain of *S. typhimurium*. A culture of this strain was mutagenized and portions containing 500 to 1,000 cells were placed on sterile Millipore filters (Millipore Corp., Bedford, Mass.) on histidine-supplemented minimal agar plates. When microcolonies had appeared, the filter was transferred to another plate lacking histidine but containing radioactive uridine. Autoradiography was used to locate colonies which had incorporated a large amount of uridine; in this way, an RC^{rel} strain was isolated. The mutation was cotransducible with the *argB* marker, the analogue of the *argA* marker of *E. coli* (60). These techniques for isolating RC^{rel} strains were used by Martin (60) and Edlin (23) to demonstrate that the RC^{rel} phenotype, which allows RNA synthesis to continue during histidine or tryptophan starvation, was not able to abolish the effects of a strong polar mutation in either the histidine or tryptophan operons.

An unexplained finding is that, despite efforts in various laboratories, it has not been possible to isolate RC^{rel} derivatives of the RC^{str} *E. coli* strain B, neither by transduction (N. Fiil, J. Gallant, *personal communication*; P. Broda, *unpublished experiments*) nor by penicillin selection (P. Donini, *personal communication*). Revertants of RC^{rel} bacteria to the RC^{str} phenotype have been reported (R. Lavallé, *in* 54). However, no RC^{str} revertants of the *E. coli* strain 58-161, carrying the classical *rel-1* mutation, have been observed.

Phylogeny of the Original rel Mutant

Strain W6 (RC^{rel}), in which Borek et al. (8) first observed the phenomenon of RNA accumulation during amino acid starvation, is a biotin-independent (Bio^+) revertant of the Met^- Bio^-RC^{str} strain 58-161. It is not clear whether the loss of the biotin requirement and the acquisition of the RC^{rel} phenotype are related. Other Bio^+ revertants of the original 58-161 strain (2; P. Broda, *unpublished results*) have retained their RC^{str} character. Hence, there does not seem to be a necessary connection between the $Bio^- \rightarrow Bio^+$ and the $RC^{str} \rightarrow RC^{rel}$ mutations. One unexplained coincidence does exist, however. It has

been observed (P. Broda, *unpublished data*) that both strain 58–161 (RC^{str}) and strain W6 (RC^{rel}), excrete glutamate on methionine starvation whereas, in other strains of *E. coli*, only RC^{rel} bacteria excrete glutamate; the corresponding RC^{str} strains do not. In other bacterial species (18), the property of glutamate excretion is almost invariably associated with the requirement for biotin, so that it is possible that there is some relation between these properties.

Dominance

It has been shown (N. Fiil, *personal communication*) that the rel^+ allele is dominant over the mutant *rel* allele. This was demonstrated by constructing partial diploids by using an F-prime factor carrying the *rel* locus. Fiil has shown that the phenotype of partial diploids of the genotypes $rel/Frel^+$ and $rel^+/Frel$ is RC^{str}; RNA synthesis is as stringently controlled as in the normal haploid rel^+ strain. Experiments to measure the kinetics of expression of the RC^{str} and RC^{rel} phenotypes are in progress. Clearly this type of information will be of great value for the evaluation of hypotheses on the nature of the function of the *rel* gene.

This system has also been used (N. Fiil, *personal communication*) to test for complementation between different RC^{rel} mutants. In the few cases that have so far been tested, such complementation has failed to produce the RC^{str} phenotype.

ROLE OF TRANSFER RNA IN THE REGULATION OF RNA SYNTHESIS

Stent and Brenner (94) postulated the intervention of amino acids in the regulation of RNA synthesis based on genetic and physiological studies of RC^{str} and RC^{rel} bacterial strains. In essence, they suggested that any of the complete set of tRNA molecules could function as inhibitors of RNA synthesis when uncharged with amino acids, but that on amino acylation the tRNA molecule was no longer inhibitory. Thus, a sufficiency of amino acids results in complete charging of the transfer RNA, whereas a deficiency would cause one or more tRNA species to become uncharged. This uncharged tRNA could then repress RNA synthesis by inhibition of the RNA polymerase. This model implicitly contains the concept of coordinate regulation of RNA synthesis, since it envisages a general reduction in the synthesis of all classes of RNA.

Kurland and Maaløe (48) presented a similar model based primarily on the effect of chloramphenicol on RNA synthesis. Owing to the importance of chloramphenicol in the development of the ideas concerning the regulation of RNA synthesis, some of the results obtained with this and other antibiotics will be discussed. At relatively low concentrations, chloramphenicol permits some residual protein synthesis; with such treatment, RNA is maximally stimulated in auxotrophic strains if catalytic amounts of required amino acids are present in the medium (74). In contrast, at higher concentrations of chloramphenicol which completely inhibit protein synthesis, the presence of exogenous amino acids is no longer required for maximal stimulation of RNA synthesis (4, 5). At first glance it seems contradictory that stopping protein synthesis by amino acid starvation represses RNA synthesis in RC^{str} strains, whereas stopping protein synthesis by addition of chloramphenicol does not depress and may actually stimulate RNA synthesis. The most straightforward explanation for this paradox appears to be that RNA synthesis is stimulated, not by any direct action of chloramphenicol on the synthesis of RNA, but because it permits the intracellular build-up of amino acid pools and of aminoacylated tRNA. It is presumed that the breakdown of protein during amino acid starvation contributes to this intracellular accumulation of amino acids when *de novo* protein synthesis is prevented by chloramphenicol or other antibiotics. In fact, it has been demonstrated that tRNA becomes fully charged with amino acids when chloramphenicol is added to an amino acid-starved culture (29, 63). Morris and DeMoss (63) observed that, although both chloramphenicol and puromycin stimulated RNA synthesis during leucine starvation of an auxotroph, the leucyl-tRNA became fully charged only on addition of chloramphenicol and not on addition of puromycin. This observation led them to propose that puromycin can stimulate RNA synthesis without increasing the level of charged tRNA. However, Ezekiel and Elkins (*in preparation*) have tested a variety of antibiotics, including puromycin, for their ability to stimulate RNA synthesis and have concluded that all of the compounds tested stimulate RNA synthesis by sparing amino acids and increasing the amount of aminoacyl-tRNA in the cell. (For a more detailed analysis of these experiments, see "Regulation by Free Ribosomes," below.)

The important conclusion that amino acid regulation of RNA synthesis depends on a complete complement of aminoacyl-tRNA and not merely on free amino acids was established with bacterial mutants. Fangman and Neidhardt (30, 31) isolated a *p*-fluorophenylalanine-resistant mutant of *E. coli* that was shown to have an altered phenylalanyl-tRNA synthetase. This strain was unable to activate *p*-fluorophenylalanine and hence to incorporate the analogue into protein.

Upon phenylalanine-starvation of this mutant strain, addition of the analogue not only did not restore protein synthesis but also did not restore RNA synthesis (27, 31). This result suggested that not only the presence of amino acids, but their activation and attachment to tRNA, is required for the promotion of RNA synthesis. This conclusion was confirmed by the isolation of temperature-sensitive bacterial mutants (6, 25, 69, 100), one of which was shown to possess an altered valyl-tRNA synthetase which is active at low temperature (30 C) but inactive at high temperature (42 C). At the high temperature, an RC^{str} strain carrying this mutation was unable to synthesize RNA, although all required amino acids were present, whereas an RC^{rel} strain carrying the same mutation was able to synthesize RNA at the high temperature. Hence it follows that an essential step in the regulation of RNA synthesis by RC^{str} strains is the attachment of amino acids to tRNA. If one constructs an RC^{rel} strain that carries the same temperature-sensitive synthetase mutation, RNA synthesis continues at the normal rate after protein synthesis is stopped by shift of the culture to the nonpermissive temperature (42 C). This result suggests that the *rel* mutation allows RNA synthesis to proceed in the absence of protein synthesis without requiring the amino acylation of tRNA. This conclusion is strengthened by the failure of chloramphenicol to appreciably stimulate RNA synthesis in a temperature-sensitive synthetase RC^{str} strain at the high temperature (Ezekiel and Elkins, *in preparation*). This observation constitutes the only clear difference between the mechanism of stimulation of RNA synthesis by chloramphenicol and the *rel* gene in the absence of protein synthesis. It was also possible to demonstrate with these mutants that repression of the enzymes of the valine biosynthetic pathway depends not on free valine but on the supply of valyl-tRNA (24). Thus, it appears that aminoacylated tRNA may be important in the regulation of diverse cellular processes.

With these results in mind, the discussion of the original hypotheses of Stent and Brenner (94) and of Kurland and Maaløe (48) can be continued. The model gained support from the experiments of Tissières et al. (99), who demonstrated that in vitro, uncharged tRNA was a considerably better inhibitor of the RNA polymerase than charged (aminoacylated) tRNA. These authors concluded that regulation of RNA synthesis might occur by inhibition of the RNA polymerase by uncharged tRNA (40, 99). Subsequent studies, however, cast doubt on the correctness of this model. Bremer et al. (9) made a more detailed examination of the inhibition of the RNA polymerase by charged and uncharged tRNA in vitro. They found that prior reaction of the RNA polymerase with deoxyribonucleic acid (DNA) protects it against subsequent inhibition by tRNA and that tRNA molecules were heterogeneous with respect to RNA polymerase inhibition. Moreover, the difference in inhibition between charged and uncharged tRNA was so small that it seemed unlikely that it could account for the observed in vivo regulation of RNA synthesis.

Ezekiel and Valulis (29) were able to increase the internal cellular tRNA concentration four- to fivefold by incubating amino acid-starved bacteria with chloramphenicol, which allows tRNA and ribosomal RNA (rRNA) to accumulate. After this treatment, the rate of RNA synthesis in these bacteria and in a control culture, which had no prior chloramphenicol treatment, was varied by starvation for a required amino acid in the presence of different amounts of chloramphenicol. For any given rate of RNA synthesis, the concentration of uncharged tRNA was four- to fivefold higher in the chloramphenicol-pretreated culture than in the control. This suggested that uncharged tRNA does not regulate RNA synthesis by inhibition of the RNA polymerase.

Differences in the intracellular condition of tRNA from RC^{str} and RC^{rel} strains have been examined in some detail. Initial experiments were designed to test the specificity of the attachment of amino acids to tRNA in extracts prepared from RC^{str} and RC^{rel} strains (59). From these extracts, purified tRNA and activating enzymes were prepared and used in different combinations to detect any break-down in the specificity of the transfer reaction. No breakdown in specificity was detected; it was therefore concluded that the *rel* gene does not affect this reaction. Further experiments explored the intracellular condition of tRNA with respect to the degree of esterification in vivo of its terminal adenosyl 3′(2′) hydroxyl with amino acids. These experiments showed that about 70% of the tRNA molecules are esterified in vivo; no significant differences were found between RC^{str} and RC^{rel} strains (101).

Differences among each of the components necessary for RNA synthesis in RC^{str} and RC^{rel} strains have also been sought by Olenick and Hahn (73) by in vitro studies. They prepared extracts of DNA, tRNA, and RNA polymerase from RC^{str} and RC^{rel} bacteria and used these extracts to stimulate RNA synthesis in an in vitro system. Different combinations of the extracts (for instance DNA and tRNA from RC^{str} bacteria and RNA polymerase from RC^{rel} bacteria) were added to an in vitro system. RNA synthesis was stimulated to the same extent by all of the

combinations tested, so it was concluded that there were no gross differences among these components in RC^{str} and RC^{rel} bacteria.

Finally, an important observation regarding the role of aminoacylated tRNA in RNA synthesis has been made by inhibiting protein synthesis with trimethoprim. Trimethoprim has been shown to inhibit the enzyme dihydrofolate reductase (11); this results in the cell's inability to synthesize *N*-formylmethionyl-tRNA. Since this particular species of tRNA is required for peptide-chain initiation, trimethoprim prevents protein synthesis in bacteria (26). More importantly for this discussion, trimethoprim has been shown to stop RNA synthesis in RC^{str} strains but not in RC^{rel} strains (90). This observation is significant because it implies that RNA synthesis depends not only upon the aminoacylation of all species of tRNA, but on some reaction subsequent to the attachment of amino acids to tRNA, since the formylation of the methionyl-tRNA occurs after attachment of methionine to its cognate tRNA. The main conclusion to be drawn from these experiments is that RNA synthesis in amino acid-starved RC^{str} strains depends either directly on *N*-formylmethionyl-tRNA or, as seems more likely, on one of the later steps in protein synthesis, which cannot occur if peptide-chain initiation is prevented.

Other Proposals Concerning the Mechanism of Action of the *rel* Locus

Polyamines

A series of studies (13, 14, 76, 77) have led S. S. Cohen and his co-workers to propose that the polyamines putrescine and spermidine have a causal role in the control of RNA synthesis. Their initial studies were made with the RC^{str} 15TAU$^-$ strain of *E. coli*, which requires thymine, arginine, and uracil for growth (47). When this strain was starved for arginine (or glucose), thereby halting RNA synthesis, there was a cellular accumulation of putrescine but not of spermidine (76). In contrast, in the presence of chloramphenicol, which permits continued RNA synthesis in the absence of arginine, a situation analogous to arginine starvation of an RC^{rel} strain, spermidine accumulates but putrescines does not. It was also observed that the addition of spermidine to the medium can stimulate RNA synthesis in the arginine-starved RC^{str} strain, and that this stimulation could be prevented by the concomitant addition of putrescine.

In a subsequent report (13), polyamine synthesis was studied in an RC^{rel} derivative of strain 15TAU, which was compared to the parent RC^{str} strain. It was reported that on arginine starvation of the RC^{rel} strain, but not of the RC^{str} strain, spermidine accumulated, and that this accumulation inhibits putrescine biosynthesis. Furthermore, during normal growth, the level of spermidine was found to be somewhat higher in the RC^{rel} strain than in its RC^{str} parent, although the amount of RNA was also found to be higher in the RC^{rel} strain than in the RC^{str} parent strain. A further observation was that on uracil starvation of the RC^{rel} strain, but not the RC^{str} strain, spermidine also accumulated. This result was interpreted to mean that spermidine accumulation does not necessarily depend on continued RNA synthesis, although the intracellular accumulation of free spermidine is correlated with RNA accumulation. As a result of these studies, it was suggested that a relaxed strain may be one in which putrescine biosynthesis is inhibited by spermidine.

The comparison between the accumulation of polyamines and RNA synthesis was extended in a later report (77). For these studies, the TAU RC^{str} strain was compared to a K-12 RC^{rel} derivative. A parallelism was demonstrated between the intracellular accumulation of spermidine and RNA under a wide variety of growth conditions. Measurements were made under conditions of normal growth, methionine stimulation of RNA synthesis, starvation for an amino acid or thymine, and inhibition by chloramphenicol or streptomycin. The general conclusion from these studies was that RNA synthesis is regulated by appropriate concentrations of spermidine and putrescine.

The stimulation of RNA synthesis by addition of spermidine to an arginine-starved RC^{str} strain appears similar to that already described for a variety of antibiotics such as chloramphenicol, streptomycin, tetracycline, etc. However, no measurements of the effect of polyamine-addition on protein synthesis were reported in the initial study (76). It has been found (Edlin and Stent, *unpublished data*) that addition of spermidine to a growing bacterial culture caused a marked reduction in the rate of protein synthesis. Similar observations were made by Ezekiel and Brockman, who examined the effects of spermidine on RNA synthesis in some detail (28). They measured the level of arginine-charged tRNA after the addition of spermidine to arginine-starved RC^{str} bacteria in amounts sufficient to stimulate RNA synthesis. They found that the level of arginine-charged tRNA increased as the stimulation of RNA synthesis increased. They also demonstrated that addition of spermidine to amino acid-starved bacteria enhances protein turnover, thereby increasing the intracellular avail-

ability of amino acids. Their findings support the idea that spermidine stimulates RNA synthesis in amino acid-starved RC^{str} strains by the sparing of amino acids and not by a more direct involvement in the regulatory process.

Against this view are the data of Raina, Jansen, and Cohen (77), who have shown that when spermidine is added to an arginine-starved culture of strain TAU RC^{str} to a concentration of 40 mM, RNA synthesis is stimulated, as measured by radioactive uracil incorporation, whereas protein synthesis also increases slightly, as measured by radioactive leucine incorporation. They infer from this result that the stimulation of RNA synthesis can occur without a corresponding intracellular buildup of the amino acid pool. Difficulties arise in comparing experiments that involve the use of spermidine because of the strong dependence on concentration and a sharp dependence on the *p*H of the medium being used.

Raina and Cohen (76) also observed that radioactive uracil incorporation into RNA continued for a considerably longer period when RNA synthesis was stimulated by addition of spermidine rather than by chloramphenicol. This finding might be explained if spermidine is able to stabilize RNA and thereby retard its breakdown. Furthermore, Lazzarini and Santangelo (52) have measnred RNA synthesis and accumulation in *B. subtilis* during chloramphenicol inhibition of protein synthesis and have concluded that the failure to accumulate RNA for more than 30 to 40 min after addition of chloramphenicol results not from the cessation of RNA synthesis but from the rapid breakdown of ribosomal RNA in the presence of chloramphenicol. It is possible that a similar situation obtains during chloramphenicol treatment of *E. coli* strains (71).

As discussed earlier, glutamate is excreted into the growth medium during arginine starvation of the RC^{rel} 15TAU$^-$ strain but not in the RC^{str} strain (P. Broda, *in preparation*). Since spermidine is also excreted by the RC^{rel} strain, it is possible that the excretion of the two substances is related. However, during uracil starvation, spermidine appears to accumulate only in the RC^{rel} strain, whereas glutamate accumulates in both the RC^{str} and RC^{rel} 15TAU$^-$ strains (P. Broda, *in preparation*). This result makes it difficult to draw any conclusions concerning the relation between glutamate and spermidine accumulation and the regulation of RNA synthesis in RC^{rel} strains.

In some respects, the choice of the 15TAU$^-$ strain for these experiments is unfortunate, because of the biosynthetic interconnections of arginine, uracil, glutamate, and polyamines. As shown by Morris and Pardee (66), putrescine, which is a precursor for spermidine, can be synthesized from either ornithine or arginine. Since the 15TAU$^-$ strain requires arginine, it is possible that polyamine synthesis in this strain is affected by the presence of arginine during growth and its subsequent removal. Moreover, glutamate is converted to glutamic semialdehyde and then to ornithine, so that glutamate accumulation in these strains may also contribute to spermidine accumulation. The manner in which the synthesis of these compounds is regulated and interconnected in RC^{str} and RC^{rel} strains of bacteria grown under different physiological conditions remains to be clarified before the correlation between their accumulation and the regulation of RNA synthesis can be understood.

Substrate Regulation of RNA Synthesis

It has been pointed out by several authors (12, 21, 33, 37) that RNA synthesis might be regulated by the supply of its nucleotide precursors. The detailed investigation of this hypothesis has become possible largely as a result of two developments. The first was the elucidation of the mechanism of RNA synthesis by RNA polymerase from a DNA template. These in vitro studies established that the ribonucleoside triphosphates were the substrates for RNA synthesis; the discussion that follows assumes that they are the immediate precursors for RNA polymerization.

The second advance has been in the development of ion exchange thin-layer chromatography (79, 80) as a technique for the rapid and quantitative separation of nucleotides. This method has been extensively used to investigate changes in bacterial nucleotide pools (12, 21). Operationally, nucleotide pools have been defined as acid-soluble material (i.e., small molecules) that diffuse freely from a bacterium when its membrane is made permeable by either acid or detergent treatment. It is possible that there is compartmentalization of nucleotides into "private pools" (10); if all the acid-soluble pools are indeed not available for RNA synthesis, then the significance of any changes in the nucleotide precursor pools becomes difficult to interpret. However, it has also been argued that it is unnecessary to postulate compartmentalization (72, 86); in this discussion, we shall assume that all nucleotides are available for nucleic acid synthesis.

Goldstein et al. (39) measured changes in nucleotide pools in a strain of *E. coli* and found that, in general, nucleotide pools tended to increase during leucine starvation. These analyses were made by the rather cumbersome techniques of column chromatography and electrophoresis. By using thin-layer chromotography, Edlin and Neuhard (21) measured changes in the nucleoside

triphosphate pools in an isogenic pair of RC^{str} and RC^{rel} strains. They reported a gradual decline in all triphosphate pools to approximately half the initial levels after 1 hr of amino acid starvation in the RC^{str} strain, but in the RC^{rel} strain there was a gradual rise in all the triphosphate pool levels, which approximately doubled after a similar period of starvation. Since the rate of RNA synthesis fell to a few per cent of the exponential rate within about 1 min at 37 C after removal of a required amino acid in an RC^{str} strain, and no change in nucleoside triphosphate pool levels was observed for at least 10 min after removal of the amino acid, it was concluded that it was unlikely that the dramatic reduction in the rate of RNA synthesis could be attributed to a lack of substrates necessary for RNA synthesis.

An important difference between RC^{str} and RC^{rel} strains was noted. During amino acid starvation, RC^{str} strains become refractory to the assimilation and phosphorylation of pyrimidines, and, to a lesser extent, of purines from the medium. RC^{rel} strains, which continue to synthesize RNA in the absence of the required amino acid, are able to incorporate purines and pyrimidines from the medium in normal fashion. No evidence was presented to indicate a causal relation between the failure to take up and phosphorylate uracil from the medium and the inability to synthesize RNA.

Gallant and Cashel (37) re-examined the effect of amino acid starvation on nucleotide pools by using bacteria which were rendered permeable to phosphorylated compounds by plasmolysis in hypertonic sucrose (41). When these sucrose-shocked bacteria were incubated with nucleoside triphosphates, radioactive uridine triphosphate (UTP) was incorporated into RNA in RC^{str} strains in a manner that was only weakly dependent on the presence of amino acids. The incorporation of radioactive uracil or uridine monophosphate (UMP), however, was more strongly amino acid dependent in RC^{str} strains than in RC^{rel} strains. These authors proposed that the regulation of RNA synthesis is effected by regulation of UTP synthesis.

These experiments are difficult to interpret in a conclusive manner for several reasons. The bacterial population is probably heterogeneous with respect to the degree to which plasmolysis has affected the metabolism of different individuals in the population. Indeed, the rate of RNA synthesis in these bacteria after plasmolysis is only about 5% of the rate in exponentially growing bacteria, which is characteristic of the rate of RNA synthesis usually obtained in vitro. One prediction of the model proposed by Gallant and Cashel was that, since synthesis of UTP was inferred to be amino acid dependent in RC^{str} but not in RC^{rel} strains, it might be expected that the UMP or uridine diphosphate (UDP) kinase reaction would be amino acid dependent in extracts from RC^{rel} strains. Such an amino acid dependence was looked for in vitro; but no significant differences were detected (J. Gallant, *personal communication*). However, it is possible that nucleoside triphosphate synthesis could be affected by mechanisms other than by an alteration of the nucleotide kinase reaction.

Subsequently, Cashel and Gallant examined the amino acid dependence of nucleoside triphosphate formation in RC^{str} and RC^{rel} strains (12) and, from the observed differences in the two strains, concluded, in contrast to the earlier conclusion of Edlin and Neuhard (21), that RNA synthesis was regulated by nucleoside triphosphate synthesis. They reported that synthesis of nucleoside triphosphates is reduced in RC^{str} strains in the absence of amino acids and is unaffected in RC^{rel} strains. In addition, they showed that the levels of UTP and cytidine triphosphate (CTP) fall rapidly to about half their normal levels in an RC^{str} histidine-requiring strain at about the same time that histidine was exhausted from the medium. They have also tried to establish a causal relationship between RNA synthesis and triphosphate synthesis by measuring the capacity of RC^{str} strains to take up and phosphorylate uracil under conditions where RNA synthesis has been blocked by actinomycin or proflavine. These experiments demonstrate that RC^{str} strains maintain normal triphosphate synthesis if RNA synthesis is prevented by means other than amino acid starvation. They again concluded from this series of experiments that RC^{str} strains respond to amino acid deficiency by limiting triphosphate synthesis, and that this is the cause of the reduction of RNA synthesis. They have suggested (12) that it is phosphorylation per se which is affected by the state of the *rel* gene.

The discrepancy between the abrupt fall in UTP and CTP pool levels in an RC^{str} histidine auxotroph upon exhaustion of histidine from the medium, reported by Cashel and Gallant (12), and the slight changes reported by Edlin and Neuhard (21) could result from differences in experimental procedure. In one case, amino acid starvation was produced by filtration and resuspension of the culture (21), and in the other case it was achieved by growth in limiting histidine (12). In addition, starvation was for histidine in one instance and for arginine or leucine in the other.

As mentioned earlier, RNA synthesis can also be prevented in RC^{str} strains by shifting a bacterial strain which has a temperature-sensitive activating enzyme from growth at low tempera-

TABLE 2. *Triphosphate pool levels after inactivation of the temperature-sensitive valine-activating enzyme*[a]

Triphosphate	NP 29 (RC^{str})				10B6 (RC^{str})			
	0 min[b]	10 min	20 min	40 min	0 min	5 min	10 min	20 min
ATP	.53	.72	.45	.28	.81	1.04	.98	.89
CTP	.10	.24	.20	.18	.22	.30	.26	.23
GTP	.20	.13	.082	.077	.27	.23	.22	.18
UTP	.14	.23	.23	.23	.36	.32	.30	.30

[a] A culture of each strain was grown for several generations in minimal glucose medium plus the required supplements and radioactive phosphate. The cultures were transferred from growth at 30 to 42 C, and portions of the culture were removed for triphosphate pool analysis at the times indicated. All portions were collected and analyzed as described in Edlin and Neuhard (21). All values are given as millimicromoles per 5×10^8 bacteria. We are grateful to F. C. Neidhardt for supplying strain NP29 and to S. Kaplan for strain 10B6.

[b] Time after shift to 42 C.

ture (30 C) to a nonpermissive temperature (42 C). The situation at the high temperature is analogous to that created by amino acid starvation but is more specific in that RNA synthesis is blocked by alteration of a single enzymatic function, rather than by the metabolically more drastic procedure of amino acid withdrawal. Table 2 illustrates the changes that occur in the triphosphate pools in two distinct *E. coli* strains, each with a temperature-sensitive valine-activating enzyme, after a shift from 30 to 42 C. In one strain, the pyrimidine triphosphate pools rise appreciably; in the other strain, the triphosphate pools appear to remain constant or fall slightly. If a culture of the parent wild-type strain without the temperature-sensitive lesion is subjected to the same shift from 30 to 42 C, all the nucleoside triphosphate pools immediately rise slightly, presumably in response to the increased growth rate at the higher temperature.

These results make it seem unlikely that RNA synthesis stops during amino acid starvation due to substrate limitation. However, the only triphosphate pool which fails to show an increase in either of the experiments presented is guanosine triphosphate (GTP) and, in fact, the GTP pool declines in each instance. On the basis of the data presented here, we would suggest that RNA synthesis is not regulated by substrate availability. However, these data leave open the possibility that GTP is involved in the amino acid regulation of RNA synthesis. The changes in the GTP pool level are not so great as to make it likely that it could block RNA synthesis by substrate limitation. However, it is conceivable that a slight decrease in the intracellular GTP concentration could prevent RNA synthesis through its role in protein synthesis. It has been reported, for example, that GTP is required for binding of aminoacyl-tRNA to ribosomes (53). If, as suggested more recently by Cashel and Gallant (12), the primary effect of amino acid-starvation of RC^{str} strains is to hinder general phosphorylation, then it may be possible to formulate a theory in which the pools of nucleoside triphosphates would remain at normal levels under conditions in which stable RNA synthesis was specifically inhibited.

Regulation by Free Ribosomes

The regulation of RNA synthesis by the intracellular concentration of free ribosomes has been suggested by several investigators (62, 64, 84). In their models, it is envisaged that amino acid starvation of an RC^{str} strain results in the breakdown of polysomes or that it prevents their formation, thereby raising the level of free ribosomes, and that these free ribosomes repress RNA synthesis in an unspecified manner. According to this model, the RC^{rel} allele would allow polysome formation in the absence of amino acids, thus preventing the accumulation of free ribosomes, so that RNA synthesis can continue.

In certain strains of *E. coli*, addition of methionine to the growth medium causes a stimulation of RNA synthesis, while leaving the rate of protein synthesis unchanged (62, 83). Matchett has also shown (62) that methionine stimulates the maturation of "chloramphenicol particles" into mature ribosomes after the removal of chloramphenicol from the culture. He suggests that this enhanced maturation of ribosomes permits their incorporation into polysomes and results in a decrease in free ribosomes; because of a feedback mechanism of the type outlined above, this decrease in free ribosomes would then allow RNA synthesis to proceed. Methionine stimulation of RNA synthesis also results in a significant increase in the accumulation of cellular spermidine, as shown by Raina, Jansen, and Cohen (77). However, since methionine is also a polyamine precursor, it is difficult to distinguish cause and effect in these experiments.

It is not clear that there is any connection between the small stimulation of RNA synthesis by methionine observed in these studies and the effect of the *rel* gene on RNA synthesis. However,

similar conclusions on the regulation of RNA synthesis by free ribosomes have been drawn from studies on polysome formation in amino acid-starved RC^{str} and RC^{rel} strains (64, 84). It was shown that, during amino acid starvation of an RC^{str} strain, there is conversion of polysomes to monosomes (64). These monosomes are rapidly reformed into polysomes if either the required amino acid or chloramphenicol is added to the culture. In contrast, polysome formation in the RC^{rel} strain is unaffected by amino acid starvation and remains at the same level as in exponentially growing bacteria. It was concluded from these observations that a high level of free ribosomes (that is, ribosomes not engaged in protein synthesis), present in amino acid-starved RC^{str} bacteria but not in amino acid starved RC^{rel} bacteria, inhibits RNA synthesis. Morris and DeMoss also noted (64) that polysomes apparently reformed in amino acid-starved RC^{str} strains on addition of chloramphenicol more rapidly than could be accounted for by de novo synthesis of messenger RNA (mRNA). They concluded that mRNA was already present in these cells during starvation, and therefore that regulation of RNA synthesis is not co-ordinate. This aspect of RNA regulation will be discussed in a later section.

More recently, however, Friesen (35) re-examined polysome formation in several strains of *E. coli* in exponential growth and on amino acid starvation. He found that the extent of breakdown of polysomes in amino acid-starved RC^{str} strains depends both on the bacterial strain used and the amino acid withheld, so that the nature of the connection between the inhibition of RNA synthesis and disappearance of polysomes is not clear.

Hartwell and McLaughlin (45) have isolated temperature-sensitive isoleucyl-tRNA synthetase mutants of yeast. As in *E. coli*, a shift to high temperature results in cessation of RNA and protein synthesis with no appreciable change in polysome content (Hartwell and McLaughlin, *personal communication*). This observation also casts some doubt on the existence of a necessary connection between the cessation of RNA synthesis and disappearance of polysomes.

The idea that the regulation of RNA synthesis is mediated by polysomes or by free ribosomes, rather than by tRNA, was tested by Ezekiel and Blumenthal (*in preparation*). They postulated that, if starvation for different amino acids results in the formation of different specific inhibitors (for instance, in the form of charged or uncharged tRNA molecules), these different inhibitors might inhibit RNA synthesis with different efficiencies. They reasoned that partial starvation for different amino acids would then produce different relationships between the rates of synthesis of RNA and of protein. They therefore compared the rates of RNA synthesis and protein synthesis under conditions in which protein synthesis was partially blocked by the addition of amino acid analogues, each causing starvation for a different amino acid. When the rate of RNA synthesis was reduced to about 35% of the exponential growth rate, protein synthesis was in each case reduced to about 70%. This finding has been interpreted as rendering less likely the possibility that RNA synthesis is regulated by 20 different inhibitors, and as being consistent with the hypothesis of a ribosomal regulation of RNA synthesis.

However, Ezekiel and Elkins (*in preparation*) interpret other evidence as being incompatible with such a ribosomal model. They examined the capacity of a wide variety of antibiotics (chloramphenicol, chlortetracycline, puromycin, lincomycin, mikamycin A, erythromycin, pactamycin, and spectinomycin) to stimulate RNA synthesis in bacteria which were tryptophan-starved by addition of the amino acid analogue 5-methyltryptophan, and in bacteria with a temperature-sensitive valyl tRNA synthetase during incubation of these bacteria at the nonpermissive temperature, when the valyl tRNA was uncharged. All of these antibiotics stimulated RNA synthesis when amino acid starvation was effected by addition of 5-methyltryptophan, but none of them was able to stimulate RNA synthesis significantly in the temperature-sensitive mutants incubated at the nonpermissive temperature. They concluded that amino acids must be attached to tRNA to stimulate RNA synthesis, and that each of these inhibitors is able to stimulate RNA synthesis by sparing amino acids.

These authors point out that, of these antibiotics, chlortetracycline, lincomycin, and mikamycin A have been reported to inhibit the binding of tRNA to ribosomes in vitro. If, in vivo, these antibiotics are effective in promoting the accumulation of charged tRNA, and also in the prevention of binding of the tRNA to ribosomes, it would then be necessary to conclude that charged tRNA can relieve the stringent control of RNA synthesis prior to the attachment to ribosomes. However, no evidence exists on the degree to which these antibiotics are effective in vivo in preventing the binding of tRNA to ribosomes, so that these experiments do not rule out the possibility that the regulation of RNA synthesis is mediated by ribosomes.

COORDINATE REGULATION OF RNA SYNTHESIS

So far we have discussed regulation of RNA synthesis as if all classes of RNA molecules re-

spond to amino acid starvation in a coordinate manner. By coordinate regulation, we mean that the synthesis of mRNA, rRNA, and tRNA in RC^{str} strains is inhibited to the same extent by removal of amino acids. This assumption was implicit in the models originally proposed (40, 48, 94), in which it was tacitly assumed that a single RNA polymerase was responsible for the synthesis of all bacterial RNA and that uncharged tRNA molecules inhibited this polymerase activity, thereby reducing RNA synthesis. Only a single *E. coli* RNA polymerase activity has been demonstrated in vitro, but it is still possible that more than one species of RNA polymerase functions in vivo.

There is general agreement that during amino acid starvation the synthesis of rRNA and tRNA is strongly inhibited in RC^{str} strains and continues in RC^{rel} strains (17, 70). The rRNA and tRNA formed in the absence of protein synthesis in RC^{rel} strains appears to be normal, except that RNA synthesized during methionine starvation has been shown to be submethylated (56, 57). The 16*S* and 23*S* rRNA which accumulates in RC^{rel} bacteria during amino acid starvation can be matured into ribosomes when protein synthesis is restored, and the tRNA synthesized under these conditions is almost fully functional in its amino acid acceptor capacity (75).

The evidence in regard to the regulation of unstable or mRNA synthesis during amino acid starvation is considerably less clear, and, in some respects, contradictory. Initial experiments seemed to support the idea that mRNA production was generally depressed during amino acid starvation of RC^{str} strains to the same extent as that of rRNA and tRNA. Gros et al. (40) characterized the synthesis of mRNA by the loss of radioactivity from pulse-labeled RNA in the presence of dinitrophenol (DNP), an inhibitor of bacterial RNA synthesis. They reported a marked reduction of "DNP labile" material in amino acid-starved RC^{str} strains, as compared with the accumulation of "DNP labile" material in amino acid-starved RC^{rel} strains. Moreover, the RNA extracted from amino acid-starved RC^{rel} strains possessed a high activity for stimulating protein synthesis in vitro, which was not the case for RNA extracted from amino acid-starved RC^{str} strains. From a variety of experiments, it was concluded (40) that a single RNA polymerase was responsible for the synthesis of RNA and that synthesis of all classes was inhibited to the same extent in RC^{str} strains during amino acid starvation, that is, that the regulation of RNA synthesis was coordinate. The general difficulty with experiments of this nature is that the amount of mRNA is inferred from the degree of functional activity, and that this activity is assumed to be a function of the actual level of mRNA.

The question of coordinate RNA regulation was re-examined by Friesen (34). In these experiments, mRNA was assayed directly by RNA-DNA hybridization. Radioactive RNA was extracted from cultures of RC^{str} and RC^{rel} bacteria which had been pulse labeled either during exponential growth or during amino acid starvation. The results indicated that, under the two conditions, the fraction of the labeled RNA which could be characterized as mRNA by hybrid formation with DNA was essentially the same; i.e., 20 to 25% of the labeled RNA was hybridizable. Since the fraction of the labeled RNA that was characterized as mRNA seemed to be constant under all conditions, these results also support the hypothesis that the regulation of RNA synthesis is coordinate.

However, the interpretation of these hybridization experiments is not without difficulties. There are several factors that could result in a significant underestimate of the amount of mRNA synthesis during amino acid starvation as compared to the synthesis of stable RNA during starvation. A half-life of mRNA that is short compared with the length of the radioactive pulse-label could lead to an underestimate of the rate of synthesis of mRNA during amino acid starvation, as measured by the hybridization procedure described above. The marked reduction in the uptake of radioactivity (21) by amino acid starved RC^{str} bacteria could also lead to an underestimate. Moreover, abundant species of mRNA may not be fully hybridized, since the amount of DNA used in the hybridization reaction is kept small so as to avoid significant hybridization of rRNA and tRNA, which are present in large excess over mRNA. As the following experiments indicate, there is reason to believe that in fact direct hybridization experiments of pulse-labeled RNA do not reflect the true rate of mRNA synthesis in amino acid starved RC^{str} bacteria.

In an attempt to circumvent these difficulties, several investigators have measured the production of a single mRNA species, namely the mRNA produced by the tryptophan biosynthetic operon of *E. coli*. This system has the advantage that it is possible to hybridize pulse-labeled RNA to derivatives of phage ϕ80 (ϕ80 pt) in which the bacterial tryptophan operon has been incorporated into the phage genome, so that tryptophan mRNA can be specifically assayed (45). By use of tryptophan auxotrophs of RC^{str} and RC^{rel} strains, synthesis of tryptophan operon mRNA was induced by tryptophan starvation, and this mRNA was assayed by hybridization to ϕ80pt

phage DNA (22). These studies showed that on tryptophan starvation, tryptophan operon mRNA constitutes a much larger fraction of the labeled RNA in the RC^{str} strain than in the RC^{rel} strain. These results were interpreted as indicating a noncoordinate regulation of RNA synthesis in RC^{str} strains during tryptophan-starvation, that is, a preferential reduction in the synthesis of rRNA and tRNA. On the basis of similar experiments, the same conclusions have been drawn by Lavellé (51).

Stubbs and Hall (92) studied the synthesis of tryptophan operon mRNA under somewhat different conditions. Instead of inducing formation of tryptophan operon mRNA by tryptophan starvation, they constructed a pair of RC^{str} and RC^{rel} arginine auxotrophs that produce tryptophan operon mRNA constitutively, and investigated the effect of arginine starvation on its production. To analyze the amounts of tryptophan operon mRNA produced, they used the technique of competitive hybridization, which permits a better quantitation than that obtained through the use of direct hybridization. Their results showed that arginine-starvation sharply reduced the intracellular level of tryptophan operon mRNA in both the RC^{str} and the RC^{rel} strain as compared to the amounts present in exponentially growing cultures. The explanation that Stubbs and Hall advance for their findings is that there may be some general regulation of the operons of amino acid biosynthesis which overrides the regulatory role of the *rel* gene, and that these results do not bear directly on the question of coordinancy. This result also raises the possibility that the noncoordinancy observed by Edlin et al. and by Lavallé (*see above*) for the tryptophan operon might be peculiar to that operon.

A number of other experiments seem to support the idea of noncoordinate RNA regulation. From experiments described in an earlier section, Morris and deMoss (64) concluded that mRNA synthesis continues in RC^{str} strains during amino acid starvation. Morris and Kjeldgaard (65) demonstrated induction of β-galactosidase mRNA in amino acid-starved RC^{str} bacteria; this also is consistent with the idea of noncoordinate regulation.

Forchhammer and Kjeldgaard (*in preparation*) measured mRNA production in amino acid-starved bacteria by determining the activity of RNA extracts in stimulating protein synthesis in an in vitro system. They first demonstrated that RNA extracted from uracil-starved bacteria has only a few per cent of the stimulating activity of the RNA from an unstarved culture. After a preliminary uracil starvation to reduce the mRNA content of the bacteria, they restored uracil, but deprived a portion of the culture of amino acids. The in vitro stimulating activity of the RNA extracted from the amino acid-starved culture increased in an identical fashion to the nonstarved culture during the initial phase of recovery and eventually leveled off at half the value attained by the nonstarved control culture. From these results, they concluded that mRNA synthesis continues in the absence of amino acids, although it has not been shown which classes of RNA contribute to this stimulating activity. In an extension of earlier studies of Stern et al. (95), Sarkar and Moldave (88) have examined the RNA synthesized in amino acid-starved cultures of an RC^{str} strain. By analysis of this RNA with respect to sedimentation velocity in a sucrose gradient, the distribution of polysomes, the decay in the presence of actinomycin, and its base composition, they conclude that the RNA synthesized during amino acid starvation is predominantly mRNA. They concluded, therefore, that the regulation of mRNA synthesis is noncoordinate.

Nierlich (*in preparation*) pulse-labeled RNA of bacteria growing in an amino acid-supplemented medium and in bacteria which were amino acid starved. Previous difficulties with this type of measurement arose from the failure of amino acid-starved bacteria to take up and phosphorylate precursors from the medium with the same kinetics as nonstarved bacteria (21). Nierlich circumvented this difficulty by measuring the specific activity of the radioactive ribonucleoside triphosphate pool at intervals of a few seconds during the period following the addition of radioactive material. Given the specific activity of the precursor pool, it is possible to convert the radioactivity incorporated into RNA into actual quantities of RNA; the rate of RNA synthesis can then be determined. The finding was that the rate of RNA synthesis in the amino acid-starved culture is about half of the rate in the supplemented culture, although accumulation of RNA in the starved culture is only a few per cent of that in the control culture. Similar results were obtained when RNA synthesis was stopped by shifting bacteria with a temperature-sensitive valine-activating enzyme from low to high temperature. At the high temperature, the rate of RNA synthesis was again about one-half of the rate in the control culture, although accumulation of RNA had stopped. These observations are consistent with the idea of noncoordinate regulation of RNA, but it should be noted that there is no characterization of the classes of RNA which are being synthesized in these experiments.

The conclusion that mRNA synthesis can continue during amino acid starvation was also arrived at from measurements of flagellar protein

formation in *B. subtilis* and *S. typhimurium* during tryptophan starvation (20). Flagellar protein contains no tryptophan residues, so that it is possible to study its formation in cells that are tryptophan starved. As in RC^{str} strains of *E. coli*, tryptophan starvation reduced RNA synthesis by 80 to 90%; however, tryptophan starvation permits appreciable flagellar protein formation. This suggests that mRNA synthesis for these proteins is relatively unaffected, although certain reservations concerning this conclusion are in order, since evidence has been presented (55, 61) that the mRNA which codes for the synthesis of flagellar protein is stable.

Finally, Goodman and Manor (*in preparation*) have measured the relative amounts of ribosomal and soluble protein synthesized in RC^{str} and RC^{rel} strains during amino acid starvation. These measurements are made as follows: a radioactive amino acid is added to RC^{str} or RC^{rel} bacteria that have been amino acid starved for about 15 min. The bacteria are exposed to radioactivity for 30 min (protein synthesis during starvation is only a few per cent of the exponential rate), followed by a 15-min chase with excess unlabeled amino acid. The bacteria are then centrifuged, washed, and suspended in fresh growth medium. After one generation of growth, they are collected and analyzed for radioactivity present in ribosomal protein and total protein, as described by Schleif (89). These experiments indicate that, during amino acid starvation, RC^{rel} strains have a value for α (rate of ribosomal protein synthesis/rate of total protein synthesis) which is more than twice the α value which is measured during exponential growth. In contrast, the α value during amino acid starvation of an RC^{str} strain is less than half the value measured during exponential growth and less than one-fourth of the α value measured for an amino acid-starved RC^{rel} strain. These experiments suggest that the synthesis of the mRNA that codes for ribosomal protein is reduced to a greater degree during amino acid starvation of an RC^{str} strain than of an RC^{rel} strain. If the synthesis of ribosomal protein mRNA is regulated in the same manner as the synthesis of of rRNA, these experiments provide additional evidence for the noncoordinate regulation of RNA synthesis by the *rel* gene.

What can be inferred from all these experiments concerning the regulation of mRNA synthesis in RC^{str} and RC^{rel} strains? These experiments seem to support the idea that amino acid regulation of RNA synthesis by the *rel* gene is not coordinate, but pertains to the regulation of stable RNA (rRNA and tRNA). This does not imply that mRNA synthesis in RC^{str} strains is not affected by amino acid starvation. We believe that mRNA synthesis is reduced in both RC^{str} and RC^{rel} strains by amino acid starvation, but that this results from the specific repression of many operons and not because of the state of the *rel* allele. Stopping protein synthesis causes many biosynthetic intermediates to accumulate; some of these certainly act to repress expression of numerous operons. Therefore, some reduction in the overall rate of mRNA is to be expected, and the experiments cited seem consistent with this view. Ideally, the problem could be resolved by measuring mRNA synthesis for a number of specific operons. This has been done for the tryptophan operon, but for technical reasons, at present, there are not many operons which can be tested in this manner.

One can advance a teleological argument to suggest why noncoordinate regulation could confer a selective advantage. When a bacterium is suddenly deprived of an essential nutrient, such as a required amino acid, it is advantageous for it to overcome the deficiency as rapidly as possible. The bacterium with a uniformly reduced capacity for RNA synthesis would be unable to recover as effectively as a bacterium that maintains the capacity for normal mRNA production. This is the situation which obtains, for example, during tryptophan starvation, when the tryptophan biosynthetic enzymes are produced at a high rate.

DISCUSSION

Before discussing the theories that have been advanced to explain the nature of the *rel* gene function, we wish to re-emphasize two conclusions which seem adequately supported by experiment and which must be accommodated by any theory. The first is that the *rel* function pertains only to the regulation of RNA synthesis by amino acids; the manner in which RNA synthesis responds to changes in growth rate resulting from changes in carbon or nitrogen sources is the same in RC^{str} and RC^{rel} strains. The second conclusion is that only the synthesis of rRNA and of tRNA, and not that of mRNA, is regulated by the *rel* gene. It is therefore concluded that the function of the *rel* gene is to adjust the rate of rRNA and tRNA synthesis to the rate at which amino acids enter proteins. This regulation serves to adjust the level of the protein-synthesizing machinery of the cell to a level commensurate with the availability of amino acids for protein synthesis.

The theories that have been proposed to explain the amino acid regulation of RNA synthesis can be summarized as follows. (i) RNA synthesis is regulated by the level of charged or uncharged tRNA (48, 63, 94). (ii) RNA synthesis is regulated by polyamines (13, 14,76,77). (iii) RNA synthesis is regulated by the availability of nucleotide

substrates (12, 37). (iv) RNA synthesis is regulated through an obligatory coupling between protein synthesis and RNA synthesis (92, 93). (v) RNA synthesis is regulated by the supply of free ribosomes or ribosomal subunits, or by some step in protein synthesis subsequent to the attachment of amino acids to tRNA (62, 64, 84, 90).

There is as yet no compelling evidence that supports one of the above theories to the exclusion of the others; however, at least the first of these can be eliminated with reasonable certainty. The original proposal that uncharged tRNA but not charged tRNA is an effective inhibitor of the RNA polymerase enzyme is clearly not the explanation of amino acid regulation of RNA synthesis.

This assertion is based primarily on the results of experiments cited in "Role of Transfer RNA in the Regulation of RNA Synthesis," above. The degree of inhibition by tRNA of RNA polymerase activity in vitro does not seem to depend significantly on whether the tRNA is charged or uncharged. Moreover, the inhibition that is obtained does not seem great enough to account for the marked inhibition of RNA synthesis observed in vivo. Also, the amount of tRNA per cell, as well as the extent to which it is charged or uncharged, can be varied over a considerable range in vivo without affecting the normal pattern of RNA regulation by amino acids.

In "Coordinate Regulation of RNA Synthesis," above, it was concluded that the function of the *rel* gene pertains only to the regulation of the synthesis of rRNA and of tRNA. If this conclusion is correct, the hypothesis that the state of tRNA regulates the synthesis of RNA would have to be modified to explain how, on amino acid starvation, mRNA is synthesized while synthesis of rRNA and tRNA is inhibited. Two possiblities are that there is more than one species of RNA polymerase or that, during amino acid starvation, there is a selective inhibition of the RNA polymerase such that mRNA can be transcribed while rRNA and tRNA cannot be transcribed.

An important argument against the tRNA theory derives from the observation (90) that trimethoprim inhibition of protein synthesis results in the cessation of RNA synthesis in RC^{str} strains but not in RC^{rel} strains. Trimethoprim interferes with the formylation of the species of methionyl-tRNA necessary for polypeptide chain initiation; this formylation occurs after the attachment of methionine to methionyl-tRNA. Therefore, it is inferred that trimethoprim blocks protein synthesis and RNA synthesis in RC^{str} strains without reducing the levels of amino acid-charged tRNA. This would suggest that an RC^{str} strain can undergo the stringent response even in a situation in which all species of tRNA are fully charged.

The sum of these experiments seems to rule out amino acid regulation of RNA synthesis by charged or uncharged tRNA, at least by its interaction with the RNA polymerase.

The theory that RNA synthesis is regulated by polyamines is not supported by convincing data. The stimulation of RNA synthesis in amino acid-starved RC^{str} strains by the addition of spermidine to the medium has been shown to result from a sparing of amino acids rather than from a more specific and direct effect on RNA synthesis (28). Measurements of polyamine levels in RC^{str} and RC^{rel} strains were initially made in bacterial strains with metabolic interrelationships which make it difficult to correlate differences in polyamine content with the state of the *rel* gene. At present, it seems likely that any difference in polyamine synthesis in RC^{str} and RC^{rel} strains is a consequence of the difference in RNA regulation rather than its cause.

The analysis of precursor regulation of RNA synthesis is complicated by the possibility of nucleotide compartmentalization, but, even with this proviso, it seems unlikely that RNA synthesis is regulated by the supply of available substrates. In general, the changes that can be observed in the nucleoside triphosphate pools on amino acid starvation are not of great magnitude (see Table 2) in either RC^{str} or RC^{rel} strains. The one possible exception to this rule is the pool of GTP, which falls quite abruptly under certain conditions of amino acid starvation of an RC^{str} strain. If this fall in the level of GTP is the cause of the stringent response to amino acid starvation, it is unlikely that it is because of its requirement as a substrate for RNA synthesis. An alternative is that it might be involved indirectly through its requirement in protein synthesis; for example, it has been reported that GTP is required to effect binding of tRNA to ribosomes (53). It is difficult, however, to suggest any satisfying mechanism whereby the synthesis of GTP could be coupled to amino acid deprivation. Moreover, this theory in its simple form does not explain how a fall in availability of substrate could limit RNA synthesis in a noncoordinate manner.

The theory of coupled synthesis of RNA and of protein has been thoroughly discussed by G. S. Stent in two recent articles (92, 93). It should be pointed out here that noncoordinate regulation of RNA synthesis would imply that it was the synthesis of stable RNA that was coupled to protein synthesis.

The theories proposing that the regulation of RNA synthesis is effected by free ribosomes are also, in effect, suggesting that the synthesis of

stable RNA is coupled to protein synthesis. However, here the emphasis is on the role of free ribosomes as inhibitors of stable RNA synthesis. On this theory, stable RNA is synthesized only when the stable RNA already present in the cell is involved in protein synthesis.

The initial report that polysomes disappeared during amino acid starvation of RC^{str} strains but not of RC^{rel} strains lent credence to the idea of regulation by free ribosomes (64). However, the more recent experiments of Friesen (35), which show that this result varies with the bacterial strain used and the particular amino acid withheld, throw some doubt on the significance of polysome disappearance during amino acid starvation of RC^{str} strains.

The inhibition of protein and RNA synthesis by trimethoprim in RC^{str} strains argues that the regulation occurs at some step after the attachment of amino acids to tRNA. Another observation, consistent with this idea, is that starvation for any of several different amino acids results in essentially the same reduction in the rate of RNA synthesis. This suggests that regulation is not effected by an array of specific inhibitors corresponding to the different amino acids, but by a more general mechanism which becomes activated by deprivation for any amino acid. The obvious site of action for such a regulatory mechanism is the polysome.

A possible basis for the difference between RC^{str} and RC^{rel} strains, consistent with the theory of regulation by the concentration of free ribosomes, would be that in RC^{rel} strains the operon for rRNA synthesis is insensitive to the accumulation of free ribosomes, in contrast to RC^{str} strains, which are sensitive. Such a model appears to be ruled out by the important observation that the *rel*$^+$ allele is dominant over the *rel* allele. An alternative explanation is that, in amino acid-starved RC^{rel} strains, a sufficiently high proportion of the cell's ribosomes are in polysomes to allow rRNA synthesis to continue, whereas polysomes break down on amino acid starvation in RC^{str} strains. Since it has been reported (35) that polysomes do not necessarily break down on amino acid starvation of RC^{str} strains, the validity of this model is questionable. The entire model of ribosomal involvement in the regulation of stable RNA synthesis will be greatly clarified when it has been determined how rapidly the dominance of the *rel*$^+$ allele is expressed in partially diploid strains. If the RC^{str} phenotype is established very rapidly after the formation of the *rel*$^+$/*rel* diploid, it would argue that the *rel* gene product is some type of repressor of ribosome synthesis. If it is established that the RC^{str} phenotype is established more slowly, it could be interpreted to mean that the *rel* gene product is a structural component of individual ribosomes. It is conceivable that one "RC^{str}" ribosome attached to a polysome could be sufficient to allow breakdown of that polysome on amino acid starvation, thereby giving rise to the stringent response.

In summary, none of the models proposed to date is entirely satisfactory in explaining the amino acid regulation of RNA synthesis. Nothing is known concerning the specific biochemical alteration that permits RNA synthesis to continue in RC^{rel} strains. Studies are now in progress to determine if the *rel* allele causes altered GTP synthesis, phosphorylation, or ribosomal protein synthesis.

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ADDENDUM IN PROOF

This review was to have appeared in the June issue, but it was delayed because of objections raised by S. S. Cohen to the discussion of his work presented here. Since the authors decided not to make any changes of substance in response to these objections, readers are referred to a summary (14) of Dr. Cohen's own views regarding the regulation of RNA synthesis.

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